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Bis(ferrocenylimine)palladium(II) and platinum(II) complexes: synthesis, molecular structures and evaluation as antitumor agents

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Abstract

Compounds (ferrocenyl-2-furylmethyl)imine (**L1**), (ferrocenyl-2-thiophenemethyl)imine (**L2**) and (ferrocenyl-2-thiopheneethyl)imine (**L3**) were synthesized by condensation reactions and obtained in very good yields. Reactions of **L1** – **L3** with 0.5 equiv of either $PdCl_2$(cod), PdClMe(cod) or $K_2[PtCl_4]$ gave the new corresponding *trans* bis(ferrocenylimine)palladium(II) and platinum(II) complexes **1** – **9**. The compounds were characterized by elemental analysis, IR, ^{1}H and ^{13}C NMR spectroscopy. The molecular structures of **3** and **6** were determined by single crystal X-ray diffraction analysis. Both structures crystallize in monoclinic $P2_1/n$ space system. The coordination geometry around the palladium atom in complexes **3** and **6** exhibits a square planar geometry at the palladium atom. Complexes **1**, **7** and **9** were evaluated for their cytotoxic activities against human breast (MCF-7) and human ovarian (A2780) cancer cell lines, and they exhibited low cytotoxic activities but comparable to that of cisplatin.

Keywords: Ferrocenylimine; Palladium; Platinum; Molecular structures; Cytotoxicity; Cancer; Cisplatin

1.0 Introduction

Cancer is a leading cause of death in both developed and developing countries, and its burden continues to increase due to population growth, aging and adoption of cancer causing behaviours [1]. The compound *cis*-diamminedichloroplatinum(II) (cisplatin) has been an established antineoplastic agent in the treatment of various cancer cells such as testicular, ovarian and bladder [2]. However the clinical effectiveness of cisplatin is greatly limited by the drug resistance and significant side effects namely nephrotoxicity, neurotoxicity, nausea and vomiting which are caused by the reaction of cisplatin with some thiol containing biomolecules such as glutathione [3-4]. This factor requires complete avoidance of sulphur-based compounds because it destroys the drug efficacy. Since the introduction of cisplatin in cancer therapy, various metal complexes and organometallic compounds have been gaining significant importance in oncology, but there has been little molecular understanding of

their mechanisms of action [3]. As the prognosis for treatment of many forms of cancer remains poor, there is compelling need for more efficient drugs to be developed in line with cisplatin.

Ferrocenylimine complexes are well known and have been prepared and investigated mainly as catalysts for carbon-carbon coupling reactions [5-6], non-linear optical materials, electrochemical sensors, liquid crystals and nano-particles [7-8]. The advantages of these complexes include their ease of preparation, facile modification and convenience of handling [6]. Ferrocene derivatives containing atoms with good donor abilities have attracted research interest because coordination of a metal to these heteroatoms produces multicentre molecules with metals in close proximity, but in different environments, which may influence the mutual cooperation of the metals in a variety of processes [9-11]. Furthermore, some ferrocenyl complexes have also been investigated for their anti-cancer activity, and exhibited promising cytotoxic activities [12-13]. The starting point in the development of new ferrocenyl derivatives as anticancer agents could be traced back to the discovery of the ferrocenium cation as an anticancer agent [14-16]. However literature reviews show conflicting conclusions about the important features in ferrocenyl compounds which could be responsible for their cytotoxic activities [12-13].

On the other hand, anticancer activity of platinum(II) and palladium(II) complexes had for a long time been associated with their *cis*-configuration, until the first active *trans*- analogues were reported [17-20]. Furthermore, bulky analogues of cisplatin have been investigated against cisplatin resistant cells and gave significant cytotoxic activities [21,22]. The important feature in these complexes is the steric crowding around the metal centre, which shields the platinum atom and thereby permitting high selectivity in binding to DNA [22,23]. Therefore, the desirable parameters for an effective anticancer drug are solubility and bulkiness but not necessarily a *cis* configuration as earlier believed. We developed new metal complexes to probe their ability to induce apoptosis. The compounds consist of bulky *trans*- bis(ferrocenylimine)palladium(II) and platinum(II) complexes attached to a furan or thiophene group. Apoptosis induction effect of these complexes has been investigated *in vitro* against MCF-7 and A2780 cancer cell lines and the results are herein discussed. The discovery of sulphur containing thioplatin, which displayed promising antitumoral activity at comparable doses with cisplatin, without severe side effects could open up a new avenue for sulphur containing compounds in anticancer therapy [24].

2. Experimental

2.1 *Materials and Instrumentation*

All manipulations were carried out under nitrogen atmosphere using a dual vacuum/nitrogen line and standard Schlenk techniques. Solvents were dried and purified by heating at reflux under nitrogen in the presence of a suitable drying agent. All the reagents and starting materials were purchased from Aldrich and were used without any further purification. The $PdCl_2$(cod) and PdClMe(cod) were prepared following literature methods [25]. The IR spectra in solution were recorded on a Perkin-Elmer Spectrum 100 Series FT-IR instrument using nujol mulls on NaCl plates. The NMR experiments were done on a Varian XR200 MHz spectrometer. Elemental analysis was performed on Server 1112 series Elemental Analyzer at the University of Cape Town. Melting points were carried out on open capillaries using SMP 10 melting point apparatus.

2.2 *Synthesis of ligands and metal complexes*

*2.2.1 (ferrocenyl-2-furylmethyl)imine (**L1**)*

Ferrocenecarboxaldehyde (0.8490 g, 3.966 mmol) was added to a Schlenk tube charged with dry MeOH (30 ml) and the mixture stirred at room temperature. (2-furyl)methylamine (0.3923 g, 4.039 mmol) was added dropwise to the stirring mixture. The reaction was stirred at room temperature for 24 hrs, resulting in an orange solution. The solvent was removed under reduced pressure to obtain an orange solid, which was washed with water (2 x 10 ml), extracted with CH_2Cl_2 and dried over $MgSO_4$. Yield: 1.0696 g (92 %) IR (nujol cm^{-1}); ν(C=N) 1636, ν(C=C furan) 1505, ν(unsub Cp) 1104, 1008, ν(C–O–C) 1320; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.19 (s, 1H, -CH=N-); 4.68 (s, 2H, =N-CH_2-); 4.18 (s, 5H, C_5H_5); 4.61 (t, 2H, *J* = 1.4, Cp); 4.39 (t, 2H, *J* = 1.8, Cp); 7.38 (d, 1H, *J* = 3.0, furan); 6.36 (t, 2H, *J* = 3.2, furan); ^{13}C NMR ($CDCl_3$) δ 163.3 (C=N); 57.4 (=N-CH_2); 69.2 (C_5H_5); 107.2, 142.0, 110.4, (furan); 70.6 (Cp); 68.7 (Cp); Anal. Calcd for $C_{16}H_{15}FeNO$: C, 65.56; H, 5.16; N, 4.78; Found: C, 65.84; H, 5.14; N, 4.76

*2.2.2 (ferrocenyl-2-thiophenemethyl)imine (**L2**)*

The ligand was synthesized according to the procedure described for **L1** using ferrocenecarboxaldehyde (0.8343 g, 3.898 mmol) and (2-thiophene)methylamine (0.4412 g, 3.898 mmol). An orange solid was obtained. Yield: 1.1450 g (95 %) IR (nujol cm^{-1}); ν(C=N) 1634, ν(C=C thiophen) 1504, ν(unsub Cp) 1103, 1021, ν(C–S–C) 1319; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.25 (s, 1H, -CH=N-); 4.81 (s, 2H, =N-CH_2-); 4.18 (s, 5H, C_5H_5); 4.67 (t, 2H, *J* = 1.6, Cp); 4.38 (t, 2H, *J* = 1.8, Cp); 7.27 (d, 1H, *J* = 2.4, thiophen); 6.98 (t, 2H, *J* = 3.2, thiophen); ^{13}C NMR ($CDCl_3$) δ 162.6 (C=N); 69.0 (C_5H_5); 59.4 (=N-CH_2); 68.6 (Cp); 70.5 (Cp); 124.5, 126.8, 124.7 (thiophen); Anal. Calcd for $C_{16}H_{15}FeNS$: C, 62.15; H, 4.89; N, 4.53; Found: C, 62.32; H, 4.89; N, 4.92

*2.2.3 (ferrocenyl-2-thiopheneethyl)imine (**L3**)*

The ligand was synthesized according to the procedure described for **L1** using ferrocenecarboxaldehyde (0.2929 g, 1.368 mmol) and (2-thiophene)ethylamine (0.1739 g, 1.367 mmol). An orange solid was obtained. Yield: 0.3977 g (90%) IR (nujol cm^{-1}); ν(C=N) 1646, ν(C=C thiophen) 1506, ν(unsub Cp) 1104, 1016, ν(C–S–C) 1321; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.06 (s, 1H, -CH=N-); 3.71(t, 2H, *J* = 6.4, =N-CH_2-); 3.20(t, 2H, *J* = 6.6, -CH_2); 4.08 (d, 5H, *J* = 0.8, C_5H_5); 4.59 (t, 2H, *J* = 1.8, Cp); 4.34 (t, 2H, *J* = 2.0, Cp); 7.13 (dd, 1H, *J* = 2.0, thiophen); 6.88 (br, 2H, thiophen); ^{13}C NMR ($CDCl_3$) δ 161.9 (C=N); 70.3 (C_5H_5); 63.1 (=N-CH_2); 31.5 (-CH_2); 69.1 (Cp); 68.4 (Cp); 123.5, 126.8, 125.0 (thiophen); Anal. Calcd for $C_{17}H_{17}FeNS$: C, 63.17; H, 5.30; N, 4.33; Found: C, 63.03; H, 5.44; N, 3.99

2.3. *Syntheses of metal complexes*

*2.3.1 trans-Dichloridobis[(ferrocenymethylidene)(furan-2-ylmethyl)amine-κN] palladium(II) (**1**)*

To a suspension of $PdCl_2$(cod) (0.0394 g, 0.138 mmol) in a mixture of CH_2Cl_2/Et_2O (20 ml) was added a solution of **L1** (0.0801 g, 0.2732 mmol) in CH_2Cl_2 (5 ml). An orange precipitate was formed immediately. The reaction was stirred at room temperature for 12 hrs. The precipitate was filtered, washed with dry hexane (2 x 5 ml) and dried under reduced pressure to obtain an orange solid. Yield: 0.0738 g (70%); mp: 149 °C; IR (nujol cm^{-1}); ν(C=N) 1634, ν(unsub Cp) 1103, 1001, ν(C–O–C) 1321, ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.80(s, 1H, -CH=N-); 4.71(s, 2H, =N-CH_2-); 3.51(t, 5H, *J* = 0.5, C_5H_5); 4.30(t, 2H, *J* = 1.2, Cp); 4.21(t, 2H, *J* = 1.4, Cp); 7.10(t, 1H, *J* = 2.8, furan); 6.48(t, 2H, *J* = 2.8, furan); Anal. Calcd for $C_{32}H_{30}Cl_2Fe_2N_2O_2Pd$: C, 50.33; H, 3.96; N, 3.67; Found: C, 49.99; H, 3.87; N, 3.73

*2.3.2 trans-Chloridobis[(ferrocenymethylidene)(furan-2-ylmethyl)amine-κN]methyl palladium(II) (**2**)*

The complex was prepared according to the procedure described in **1**, using PdClMe(cod) (0.0567 g, 0.2139 mmol) and **L1** (0.1260 g, 0.4298 mmol). An orange solid was obtained. Yield: 0.1145 g (72%); mp: 140 °C; IR (nujol cm^{-1}); ν(C=N) 1633, ν(unsub Cp) 1105, 1008, ν(C–O–C) 1323, ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.97(s, 1H, -CH=N-); 4.59(s, 2H, =N-CH_2-); 3.49(t, 5H, *J* = 0.8, C_5H_5); 4.33(t, 2H, *J* = 1.2, Cp); 4.18(t, 2H, *J* = 1.2, Cp); 7.05(t, 1H, *J* = 2.8, furan); 6.57(t, 2H, *J* = 3.0, furan); 1.24(s, 3H, Pd-Me); Anal. Calcd for $C_{33}H_{33}ClFe_2N_2O_2Pd$: C, 53.33; H, 4.48; N, 3.77; Found: C, 53.30; H, 4.68; N, 3.80

*2.3.3 trans-Dichloridobis[(ferrocenylmethylidene)(thiophen-2-ylmethyl)amine-κN] palladium(II) (**3**)*

The complex was prepared according to the procedure described for **1**, using $PdCl_2$(cod) (0.0943 g, 0.330 mmol) and **L2** (0.2040 g, 0.660 mmol). An orange solid was obtained. Single crystals suitable for X-ray crystallography were grown by the slow diffusion of hexane into a solution of the complex in CH_2Cl_2. Yield: 0.1858 g (71%); mp: 147 °C; IR (nujol cm^{-1}); ν(C=N) 1630, ν(unsub Cp) 1105, 1002, ν(C–S–C) 1321, ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.79(s, 1H, -CH=N-); 4.65(s, 2H, =N-CH_2-); 3.47 (t, 5H, *J* = 0.6, C_5H_5); 4.31(t, 2H, *J* = 1.2, Cp); 4.23(t, 2H, *J* = 1.4, Cp); 7.14(t, 1H, *J* = 2.2, thiophen); 6.32(t, 2H, *J* = 2.4, thiophen); Anal. Calcd for $C_{32}H_{30}Cl_2Fe_2N_2PdS_2$: C, 48.30; H, 3.80; N, 3.52; Found: C, 48.25; H, 3.97; N, 3.25

*2.3.4 trans-Chloridobis[(ferrocenylmethylidene)(thiophen-2-ylmethyl)amine-κN]methylpalladium(II) (**4**)*

The complex was prepared according to the procedure described for **1**, using PdClMe(cod) (0.0667 g, 0.2516 mmol) and **L2** (0.1512 g, 0.490 mmol). An orange solid was obtained. Yield: 0.1483 g (76%); mp: 138 °C; IR (nujol cm^{-1}); ν(C=N) 1629, ν(unsub Cp) 1105, 1004, ν(C–S–C) 1320, ^{1}H NMR (200 MHz, $CDCl_3$):): δ 7.99(s, 1H, -CH=N-); 4.68(s, 2H, =N-CH_2-); 3.49(t, 5H, *J* = 0.8, C_5H_5); 4.35(t, 2H, *J* = 1.4, Cp); 4.20(t, 2H, *J* = 1.4, Cp); 7.12(t, 1H, *J* = 2.6, thiophen); 6.13(t, 2H, *J* = 2.4, thiophen); 1.26(s, 3H, Pd–Me); Anal. Calcd for $C_{33}H_{33}ClFe_2N_2PdS_2$: C, 51.12, H, 4.29; N, 3.61; Found: C, 50.84; H, 4.42; N, 3.65

*2.3.5 trans-Dichloridobis[(ferrocenylmethylidene)(thiophen-2-ylethyl)amine-κN] palladium(II) (**5**)*

The complex was prepared according to the procedure described for **1**, using $PdCl_2$(cod) (0.0209 g, 0.073 mmol) and **L3** (0.0459 g, 0.142 mmol). An orange solid was obtained. Yield: 0.0445 g (74%); mp: 146 °C; IR (nujol cm^{-1}); ν(C=N) 1632, ν(unsub Cp) 1104, 1002, ν(C–S–C) 1320, ^{1}H NMR (200 MHz, $CDCl_3$):): δ 7.76(s, 1H, -CH=N-); 4.38(t, 2H, *J* = 6.2, =N-CH_2-); 4.30(t, 2H, *J* = 6.4, -CH_2); 3.44(t, 5H, *J* = 0.5, C_5H_5); 4.25(t, 2H, *J* = 1.4, Cp); 4.14(t, 2H, *J* = 1.2, Cp); 7.09(d, 1H, *J* = 2.4, thiophen); 6.85(t, 2H, *J* = 2.6, thiophen); Anal. Calcd for $C_{34}H_{34}Cl_2Fe_2N_2PdS_2$: C, 49.57; H, 4.16; N, 3.40; Found: C, 49.50; H, 4.01; N, 3.61

*2.3.6 trans-Chloridobis[(ferrocenylmethylidene)(thiophen-2-ylethyl)amine-κN]methyl palladium(II) (**6**)*

The complex was prepared according to the procedure described for **1**, using PdClMe(cod) (0.0324 g, 0.122 mmol) and **L3** (0.0789, 0.244 mmol). An orange solid was obtained. Single crystals suitable for X-ray crystallography were grown by slow diffusion of hexane into a solution of the complex in CH_2Cl_2. Yield: 0.0686 g (70%); mp: 135 °C; IR (nujol cm^{-1}); ν(C=N) 1632, ν(unsub Cp) 1103, 1001, ν(C–S–C) 1320, ^{1}H NMR (200 MHz, $CDCl_3$):): δ 7.93(s, 1H, -CH=N-);

4.98(t, 2H, *J* = 6.0, =N-CH_2-); 4.58(t, 2H, *J* = 6.2, -CH_2); 3.73(t, 5H, *J* = 0.6, C_5H_5); 4.31(t, 2H, *J* = 1.2, Cp); 4.17(t, 2H, *J* = 1.2, Cp); 7.18(d, 1H, *J* = 2.8, thiophen); 6.74(t, 2H, *J* = 2.4, thiophen); 1.55(s, 3H, Pd-Me); Anal. Calcd for $C_{35}H_{37}ClFe_2N_2PdS_2$: C, 52.33; H, 4.64; N, 3.49; Found: C, 52.83; H, 4.59; N, 3.29

*2.3.7 trans-Dichloridobis[(ferrocenymethylidene)(furan-2-ylmethyl)amine-κN] platinum(II) (**7**)*

To a suspension of **L1** (0.0873 g, 0.298 mmol) in CH_2Cl_2 (4 ml) was added a solution of $K_2[PtCl_4]$ (0.0623 g, 0.1501 mmol) in (2:1) MeOH/H_2O (3 ml). The reaction was allowed to proceed under reflux for 6 hrs, after which it was allowed to settle. A colourless aqueous layer separated over a red organic layer. The organic layer was recovered, followed by addition of excess hexane to precipitate out a light brown solid. The solid was filtered under vacuum, washed with water and hexane and dried. Yield: 0.0578 g (69%); mp: 168 °C; IR (nujol cm^{-1}); ν(C=N) 1619, ν(unsub Cp) 1106, 1012, ν(C–O–C) 1318; ^{1}H NMR (200 MHz, $CDCl_3$):): δ 7.82(s, 1H, -CH=N-); 4.45(s, 2H, =N-CH_2-); 3.53(t, 5H, *J* = 0.8, C_5H_5); 4.30(t, 2H, *J* = 1.4, Cp); 3.82(t, 2H, *J* = 1.2, Cp); 7.08(d, 1H, *J* = 2.8, furan); 6.47(t, 2H, *J* = 2.6, furan); Anal. Calcd for $C_{32}H_{30}Cl_2Fe_2N_2O_2Pt$: C, 45.10; H, 3.55; N, 3.29; Found: C, 44.98; H, 3.68; N, 3.43

*2.3.8 trans-Dichloridobis[(ferrocenylmethylidene)(thiophen-2-ylmethyl)amine-κN] platinum(II) (**8**)*

The complex was prepared according to the procedure described for **7**, using $K_2[PtCl_4]$ (0.0697 g, 0.168 mmol) and **L2** (0.1040 g, 0.336 mmol). A light brown solid was obtained. Yield: 0.0528 g (65%); mp: 163 °C; IR (nujol cm^{-1}); ν(C=N) 1619, ν(unsub Cp) 1106, 1000, ν(C–S–C) 1317; ^{1}H NMR (200 MHz, $CDCl_3$):): δ 7.82(s, 1H, -CH=N-); 4.45(s, 2H, =N-CH_2-); 3.50(t, 5H, *J* = 0.6, C_5H_5); 4.26(t, 2H, *J* = 1.4, Cp); 3.73(t, 2H, *J* = 1.2, Cp); 7.03(d, 1H, *J* = 2.4, thiophen); 6.72(t, 2H, *J* = 2.8, thiophen); Anal. Calcd for $C_{32}H_{30}Cl_2Fe_2N_2PtS_2$: C, 43.46; H, 3.42; N, 3.17; Found: C, 43.20; H, 3.23; N, 3.03

*2.3.9 trans-Dichloridobis[(ferrocenylmethylidene)(thiophen-2-ylethyl)amine-κN] platinum(II) (**9**)*

The complex was prepared according to the procedure described for **7**, using $K_2[PtCl_4]$ (0.0589 g, 0.142 mmol) and **L3** (0.0928 g, 0.287 mmol). A light brown solid was obtained. Yield: 0.0463 g (68%); mp: 158 °C; IR (nujol cm^{-1}); ν(C=N) 1621, ν(unsub Cp) 1106, 1002, ν(C–S–C) 1319; ^{1}H NMR (200 MHz, $CDCl_3$):): δ 7.76(s, 1H, -CH=N-); 4.37(s, 2H, =N-CH_2-); 4.32(s, 2H, -CH_2); 3.22(t, 5H, *J* = 0.8, C_5H_5); 4.23(t, 2H, *J* = 1.2, Cp); 3.90(t, 2H, *J* = 1.2, Cp); 7.14(d, 1H, *J* = 2.4, thiophen); 6.89(t, 2H, *J* = 2.0, thiophen); Anal. Calcd for $C_{34}H_{34}Cl_2Fe_2N_2PtS_2$: C, 44.75; H, 3.76; N, 3.07; Found: C, 44.62; H, 3.91; N, 2.99

2.4 β-Cyclodextrin inclusion complex 1b

To an aqueous solution of β-cyclodextrin (10 ml) (0.0549 g, 0.0484 mmol) in a Schlenk tube was added dropwise an EtOH solution (5 ml) of complex **1** (0.0179 g, 0.0234 mmol). The resultant solution was stirred at 60 ^{o}C for 8 hrs, resulting in the formation of an orange precipitate. The precipitate was cooled, after which it was isolated by vacuum aided filtration, washed with MeOH (2 x 2 ml) and dried in a dessicator to obtain an orange solid. IR (nujol cm^{-1});1632, 1105, 1000

2.5 Molecular structures of 3 and 6

Single crystals of complexes **3** and **6** suitable for X-ray crystallography were grown by slow diffusion of hexane into a CH_2Cl_2 solution of the complex at 4 ^{o}C. X-ray diffraction data for compounds **3** and **6** were collected on a Bruker KAPPA APEX II DUO diffractometer using graphite-monochromated Mo-Kα radiation (χ = 0.71073 Å). The crystal structure was solved by direct methods using SHELX [26] and refined by full-matrix least-squares methods based on F^2 [26] using SHELX [26] and using the graphics interface program ORTEP-3 for Windows [27,28].

2.6 Cytotoxicity determination

2.6.1 Cell culture

The A2780 and MCF-7 cells were cultured in RPMI-1640 in 25cm tissue culture flasks and were allowed to grow to 90% confluency in an incubator set at 37 ^{o}C containing 5% CO_2 atmospheric pressure, before they were trypsinized and cultured in 6 tissue culture plates.

2.6.2 Morphological evaluation

The cells were treated with 100 μM of the complexes and incubated for 24 hours at 37 ^{o}C in 5% humidified CO_2 incubator. Following incubation, the cells were inspected under an inverted (Nikon) light microscope using 20X objective and pictures were taken using a Leica EC3 digital camera. The morphology of the cells was evaluated and recorded as per literature recommendations [29,30]

2.6.3 MTT assay

The cells were plated in 96-well tissue plates at a density of 2.0 x 10^5 cells per well and treated with various concentrations of the palladium complexes that ranged from 100 to 10μM after which they were incubated for 24 hrs. Triplicate wells were established for each concentration. Just 5 hrs before the elapse of 24 hours, 10μl of 5mg/ml MTT solution was added to each well and the plates were further incubated. At the end of the incubation period, the media was removed from each

well and replaced with 50 µl of DMSO. The plates were shaken on a rotating shaker for 10 minutes before taking readings at 560 nm using a microplate reader.

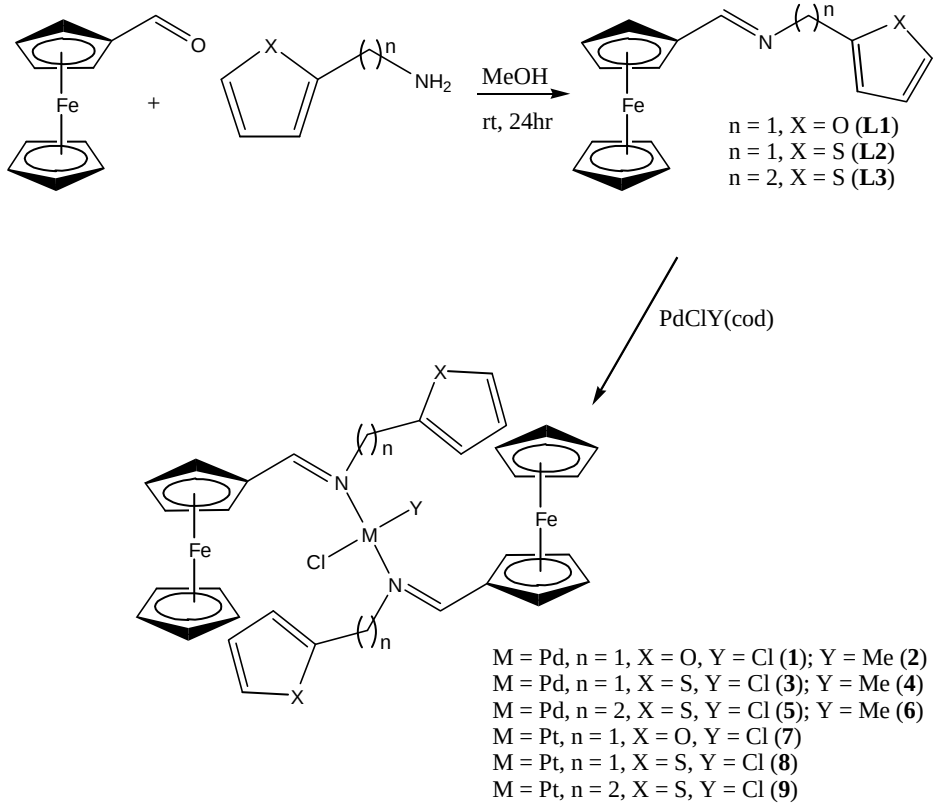
2.6.4 APOPercentage ™ apoptosis assay

The cells were plated in 96-well tissue plates and treated with 100µM of the complexes, after which they were incubated for 24 hours. The cells were also induced with 100 µM of cisplatin to save as a positive control. After incubation, the cells were washed twice with PBS and stained for 30 min with APOP*ercentage*™ dye. Staining was analyzed on a FASCan™ (Becton Dickson) instrument equipped using a 488 nm Argon Laser as a light source.

3.0 Results and discussion

3.1 Synthesis of the ligands and metal complexes

Ferrocenylimine ligands **L1 – L3** were synthesized *via* Schiff base condensation reaction of ferrocenecarboxaldehyde with an appropriate heterocyclic amine (Scheme 1). The compounds were obtained as air stable, reddish brown solids in excellent yields of over 90 %. The ligands are soluble in common organic solvents. Ligand **L1** is reported in this work for the first time to the best of our knowledge. However **L2** and **L3** have been reported in the literature [31], but were independently prepared and fully characterized by us.



Scheme 1: Preparation of *trans*-bis(ferrocenylimine) Pd(II) and Pt(II) complexes **1 – 9**

The ligands **L1 – L3** were reacted with either $PdCl_2$(cod), PdClMe(cod) or $K_2[PtCl_4]$ at room temperature to give the corresponding new *trans*-bis(ferrocenylimine)palladium(II) and platinum(II) complexes (Scheme 1). The complexes precipitated from the reaction solution, and the addition of excess Et_2O to the reaction solution increased the yields of the palladium complexes. The ferrocenylimine ligands bind monodentately to the metal atom. This coordination mode is confirmed in the crystal structures of complexes **3** and **6** (Fig.s 1 and 2). The binding is similar to that of related ferrocenylpyridine complexes reported in the literature [12].

All the compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy, IR and elemental analyses. The micro-analysis data are consistent with the proposed molecular formulas. A strong absorption band between 1634 and 1646 cm^{-1} was observed in the IR spectra of the ligands, which indicated imine formation [6,31,32]. The other strong absorption bands observed in the ligands at around 1105 and 1010 cm^{-1} show the presence of an unsubstituted cyclopentadienyl ring [31]. Coordination of the ligands to the metal centre was further confirmed from the IR spectra of complexes **1** – **9** which showed lower absorption bands between 1629 and 1634 cm^{-1} when compared to their corresponding free ligands [33].

In the ^{1}H NMR spectra of the ligands, singlet peaks were observed between 8.06 and 8.22 ppm due to the hydrogen atom in the imine (**H**C=N) moiety [31]. The signal for the methylene arm (=N-CH_2) in **L1** and **L2** appeared as a singlet at 4.68 ppm and 4.81 ppm respectively, while the two triplets observed at 3.20 ppm and 3.71 ppm in **L3** were a result of an ethylene arm present [31]. The ferrocenyl protons were observed at around 4.10 ppm. In the ^{13}C NMR analysis of the ligands, the characteristic signals for the imine carbons appeared between 161.9 ppm and 163.3 ppm [31,34]. The ^{1}H NMR spectra of complexes **1** – **9** showed imine protons in the upfield region 7.76 – 7.99 ppm, which confirmed coordination to the palladium metal centre [32]. The ferrocenyl protons in the complexes had shifted to around 3.50 ppm.

3.2 Single crystal X-ray diffraction studies

The molecular structures of complexes **3** and **6** were determined by X-ray diffraction studies. The molecular structures are shown in Figures 1 and 2 respectively. Crystallographic data and refinement residuals are summarized in Table 1 while selected bond lengths and bond angles are summarized in Table 2.

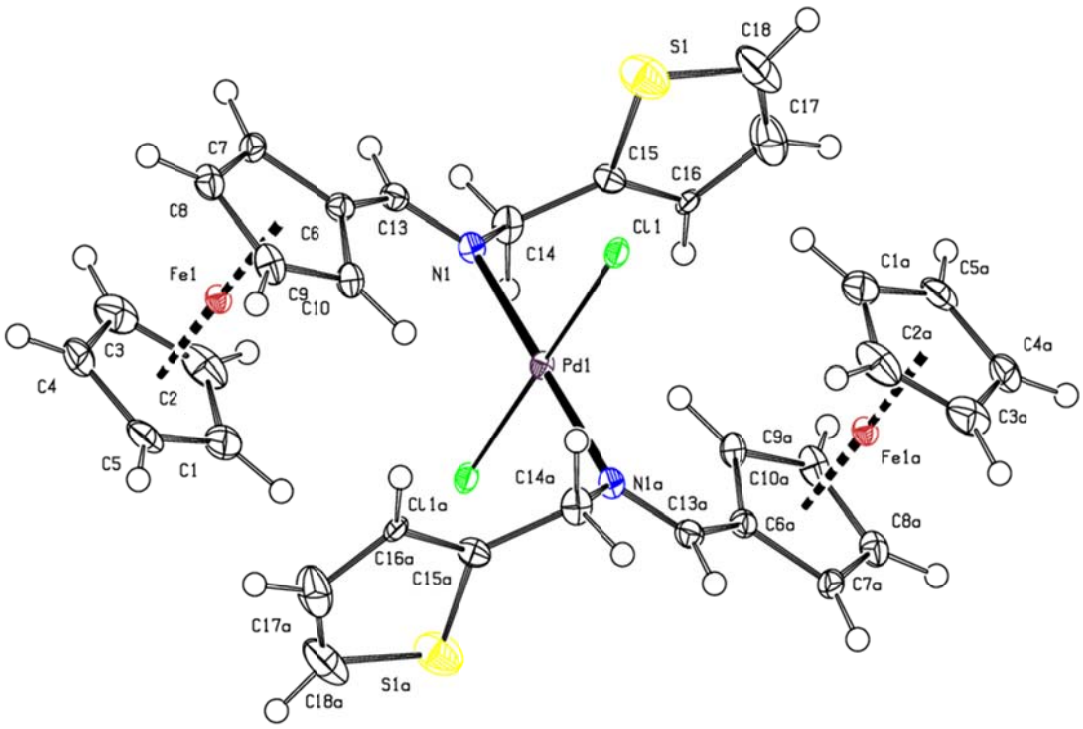


Fig. 1 X-ray crystal structure of complex **3**

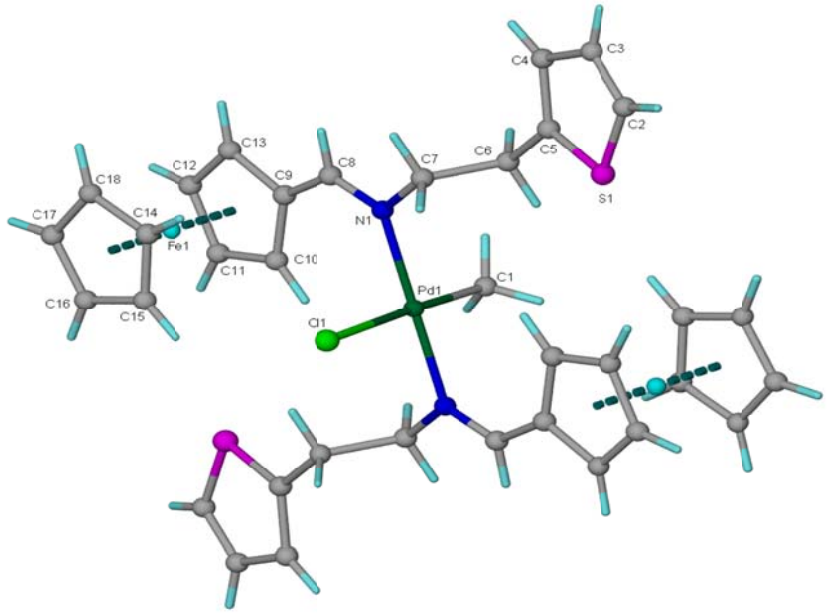


Fig. 2 X-ray crystal structure of complex **6**

Both molecules crystallize in the monoclinic space group $P2_1/n$ with two asymmetric molecules per unit cell. The two molecular structures exhibit square

planar geometry around the palladium atom. In each of the molecules, the palladium atom is coordinated to two *trans*- ferrocenylimine molecules *via* their imine nitrogen atoms, and either two chlorides or a chloride and a methyl.

Table 1: Crystallographic data and refinement for complexes **3** and **6**

Crystallographic data	**3**	**6**
Empirical formula	$C_{32}H_{30}Cl_2Fe_2N_2PdS_2$	$C_{35}H_{37}ClFe_2N_2PdS_2$
Formula weight	795.7	803.34
Temperature (K)	100(2)	173
Crystal system	monoclinic	monoclinic
Space group	$P2_1/n$	$P2_1/n$
Unit cell dimensions		
a (Å)	12.6817(12)	12.8429(5)
b (Å)	7.3785(6)	7.3412(2)
c (Å)	16.4034(15)	17.1277(7)
β (°)	102.789(2)	95.498(2)
V (Å^3)	1496.8(2)	1607.41(10)
Z	2	2
D_{cal} (Mgm^{-3})	1.687	1.660
F($_{000}$)	766	816
Absorption coefficient (mm^{-1})	1.805	1.69
Crystal size (mm^3)	0.25 x 0.04 x 0.02	0.20 x 0.13 x 0.04
Final R indices (R1)	R1 = 0.050, wR2 = 0.144	R1 = 0.037, wR2 = 0.096
Theta range for data collection	1.85 to 28.34°	3.0 to 26.4°
Goodness-of-fit on F^2	1.141	1.08
Largest diff. peak and hole	1.957 and -1.049e.Å^{-3}	0.84 and -0.60 e.Å^{-3}

Table 2: Selected bond lengths and bond angles for complexes **3** and **6**

Complex	**Bond length (Å)**		**Bond angle (°)**
3	Pd(1)–N(1)	2.031(3)	N(1)–Pd(1)–Cl(1) 92.22(11)
	Pd(1)–N(1a)	2.031(3)	N(1)–Pd(1)–Cl(1a)
	Pd(1)–C1(1)	2.388(2)	92.22(11)

	Pd(1)–C1(1a)	2.388(2)	N(1a)–Pd(1)–Cl(1) 87.78(11) N(1)–Pd(1)–Cl(1a) 87.78(11)
6	Pd(1)–N(1) Pd(1)–N(1a) Pd(1)–C1(1) Pd(1)–C(1)	2.032(3) 2.032(3) 2.418(4) 1.999(13)	N(1)–Pd(1)–Cl(1) 88.83(17) N(1)–Pd(1)–C(1) 93.80(7) N(1a)–Pd(1)–Cl(1) 91.17(17) N(1a)–Pd(1)–C(1) 86.20(7)

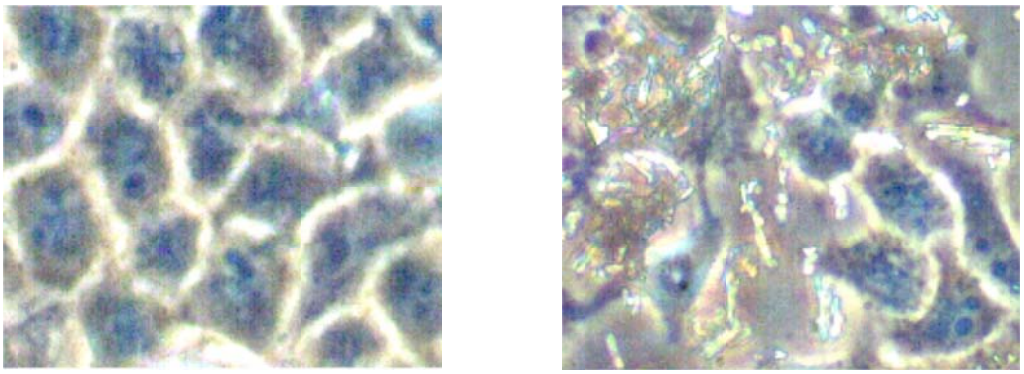
The bond angles around the palladium metal atoms of N(1)–Pd(1)–Cl(1) (92.22(11)°) and N(1a)–Pd(1)–Cl(1) (87.78(11)°) in **3** and N(1)–Pd(1)–Cl(1) (91.17(17)°) and N(1)–Pd(1)–C(1) (93.80(7)°) in **6** showed small deviations from planarity, which supports the observed geometry. Furthermore, bond angles N(1)–Pd(1)–Cl(1) of 92.22(11)° in **3** and N(1)–Pd(1)–Cl(1) of 91.17(17)° in **6** are similar. They are slightly higher than those of closely related structures [12].

However, the average Pd(1)–N(1) bond lengths of 2.031(3)Å in **3** and **6** compare well with the literature values [12]. The Pd(1)–C1(1) bond length of 2.388(2)Å in **3** is in good agreement with the average Pd–Cl bond distance of 2.298(15)Å for known palladium complexes [35]. There is detectable *trans*- influence taking place in compound **6** since the Pd(1)–C1(1) bond length of 2.418(4)Å is significantly longer than the average value [35,36]. The X-ray molecular structures of complexes **3** and **6** give conclusive evidence of their *trans*- geometry. The *trans*- geometry is also assumed for the other bis(ferrocenylimine) complexes due to their steric nature, but a *cis*- geometry is still possible for these complexes in solution [37].

3.3 Cytotoxicity studies

The aim of this study was to evaluate *trans*-bis(ferrocenylimine)palladium(II) and platinum(II) complexes as possible antitumor agents which could induce apoptosis. The complexes were chosen for two reasons: firstly, they are bulky,

and as seen from the introduction, bulky analogues of cisplatin decrease the rate of hydrolysis and substitution reactions of the cisplatin analogues by shielding the metal center and thereby permitting high selectivity to DNA binding [21-23]. This mode of action, viewed as the primary mechanism in these anticancer drugs is responsible for the increase in antitumor activity for active platinum complexes. Secondly, the complexes possess a *trans*- configuration, and this particular geometry has been less explored for its ability to exert cytotoxicity. However, literature reviews showed that the *trans*- isomers could exhibit cytotoxic activities equivalent or even better than the *cis*- isomers or cisplatin [19,20,38]. The *trans*- isomers strongly associate with DNA by long range cross-links to form 1,3-intrastrand and interstrand bifunctional adducts. The resulting adducts are capable of distorting DNA in a similar manner to 1,2-intrastrand cross-link of cisplatin. This phenomena is viewed as the reason for their effectiveness against cisplatin-resistant cell lines [3,19,39]



(a) **(b)**

Fig. 3 Sample of morphological images of **(a)** untreated and **(b)** treated **MCF7** cell lines

The ferrocenylimine ligand **L1** and *trans*-bis(ferrocenylimine) complexes **1**, **7** and **9** were evaluated *in vitro* for their cytotoxic activity against human ovarian (A2780) and highly invasive human breast (MCF-7) cancer cell lines using cisplatin as a standard. The cytotoxicity of cisplatin was evaluated under the same experimental conditions for comparison. The aim of evaluating **L1** was to assess whether free ferrocenylimine ligands could exert cytotoxicity on cancer cells as it has been observed before with other ferrocenyl ligands [40]. The compounds were not readily soluble in water, hence they were first solubilized in dimethylsulfoxide(DMSO). This is a polar aprotic solvent which is excellently useful in synthesis and formulation applications. The solvent is also effective in accelerating the rate of chloride displacement from a complex [37].

Table 3: Growth inhibition values of compounds **7** and **9** tested against **MCF7** and **A2780** cells

	Complex (IC_{50})		
Cell line	**Cisplatin**	**7**	**9**
MCF7	88	92	98
A2780	100	>100	>100

IC_{50} is the concentration of the complex required to inhibit cell growth by 50%

Cell proliferation was determined by using the MTT assay with minor modifications [40-42]. The assay measures the amount of MTT reduction by the mitochondrial dehydrogenase based on the ability of live cells that incorporate and bind the dye. The details of cytotoxicity investigation are given in the experimental section, and the results of these experiments are summarized in Table 3. The ligand, **L1** did not show any activity. Complex **1** proved to be insoluble as confirmed by the formation of crystals in the treated cells (Fig 3). This observation suggests that the complex precipitated back to its undissolved state thus limiting its bioavailability. Similarly, some complexes containing ferrocenyl ligands have also been reported and the attempt to evaluate their cytotoxicity was hindered by this insolubility phenomenon [12].

In view of the solubility problem realized from complex **1**, an attempt was made to improve its aqueous solubility through generating an inclusion complex using β-cyclodextrin. An inclusion complex presents a unique and attractive technique that enhances aqueous solubility of a poorly soluble compound. It is evident from the literature that inclusion complexes inject positive changes in potential drug properties such as enhanced solubility, physical and chemical stability [40,43,44]. This is regarded as one of the practical procedures that enhance drug dissolution rate and bio-availability. However, there was no observable shift from the stretching frequencies of the characteristics bands of complex **1** in the IR spectrum. Unfortunately, this observation indicates the absence of an inclusion complex. The result could have been due to complex **1** being sterically demanding, and thus not properly encapsulated into cyclodextrin cavity. The unsuccessful attempt to solubilize bis(ferrocenylimine) palladium(II) complexes prevented further antitumoral investigations on these complexes.

Conversely, the platinum(II) complexes displayed stability in aqueous media, thus allowing for antitumor screening. The IC_{50} values of complexes **7** and **9** for growth inhibition of MCF-7 cell lines were obtained as 92 and 98 μM respectively.

These growth inhibitory activities are very much comparable to that of cisplatin (IC_{50} 88 μM). The furan or thiophene groups on the complexes did not show any significant influence on the cytotoxic properties of the complexes.
The observed activities exhibited by the complexes confirmed the potential of *trans*- isomers to exert cytotoxicity on cancer cell lines [45]. However, the complexes exhibited very low activities against A2780 cell lines (IC_{50} >100 μM) to claim any remarkable achievement [19,46].

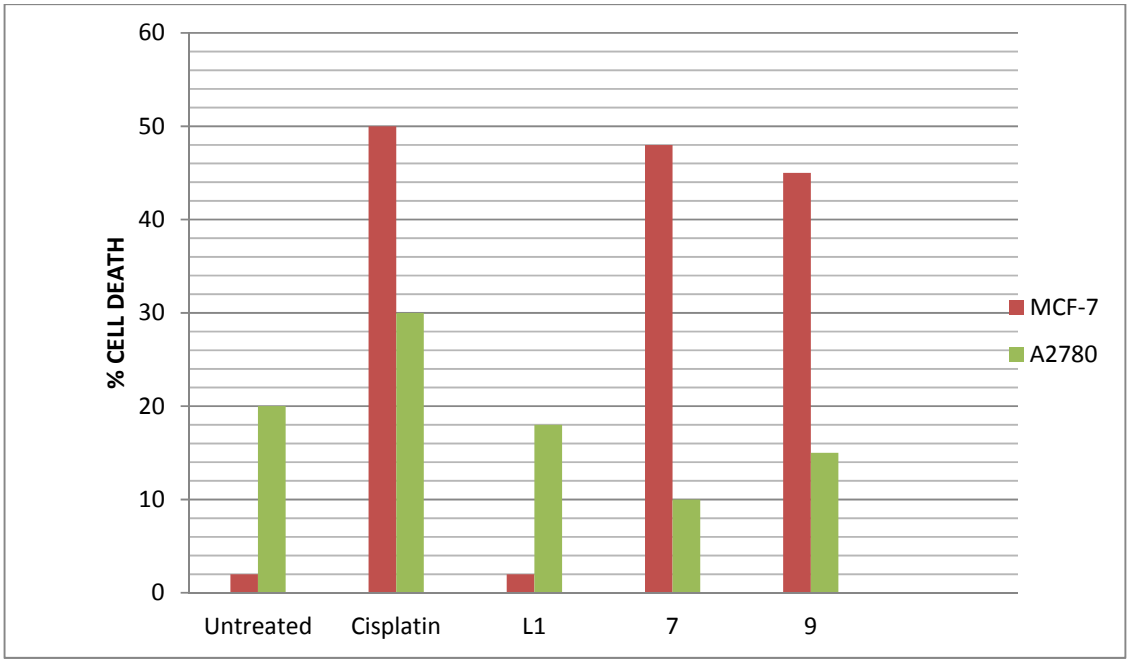


Fig. 4 Quantification of pro-apoptotic activities of complexes **7** and **9** against **MCF-7** and **A2780** cells

The uptake of APO*Percentage* dye was used to evaluate the pro-apoptotic activity of the complexes. This assay helps with detection and quantification of apoptosis. The APO*Percentage* assay was performed following a literature procedure [47]. In the pro-apoptotic activity of the complexes (Fig. 4), **7** and **9** induced cell death of around 45% on MCF-7 cancer cell lines, returning a value which is almost equal to that of cisplatin, though lower. There was no observed difference between the complexes and untreated A2780 cancer cells, suggesting that there was no pro-apoptotic activity against A2780 cancer cells by the complexes.

Conclusion

In conclusion, the new *trans*-bis(ferrocenylimine)palladium(II) and platinum(II) complexes have been successfully synthesized and fully characterized. The

complexes were evaluated for the first time for their cytotoxic activity against MCF-7 and A2780 cancer cell lines. The free ferrocenylimine ligands did not show any cytotoxic activity on cancer cells. The *trans*-bis(ferrocenylimine)palladium(II) complexes were found to be insoluble, which hindered their capacity to go through the cellular membrane. The platinum(II) complexes exhibited cytotoxic activities againt MCF-7 cancer cell lines which are comparable to that of cisplatin, but they performed poorly against A2780 cancer cells. The presence of the furan or thiophene groups on the complexes did not influence the cytotoxic properties of the complexes.

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Supplementary material
Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. **844279** and **857265** for compounds **3** and **6** respectively. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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